DEFENSE AND TOLERANCE IN ASCLEPIAS SYRIACA L. (ASCLEPIADACEAE)

BY

CRIS G. HOCHWENDER

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Abstract

This study explored the evolution of resistance for *Asclepias syriaca* L. (Asclepiadaceae). Vertebrate herbivores caused much more damage than insect herbivores. Natural levels of damage ranged from 2% to 23% among 12 genets, suggesting that herbivores may decrease plant growth and that genetic variation in defense potentially exists.

In a common garden experiment, significant genetic variation in defense against *Uromyces asclepiadis* and almost significant genetic variation in defense against *Danaus plexippus* were detected. Selection pressure by these plant enemies could lead to increased levels of defense. In addition, this experiment detected genetic variation in tolerance to damage, as some genotypes were more able to tolerate foliar damage than other genotypes.

To further investigate the potential for and constraints on the evolution of plant tolerance to herbivores, a greenhouse experiment was performed in which soil nutrient concentration was manipulated. Families differed significantly in tolerance when experiencing high nutrient conditions, and families were almost significantly different in tolerance when grown under low nutrient conditions. The interaction between family and nutrient treatment for tolerance would not constrain the rate at which tolerance could evolve under each nutrient condition. Under low nutrient conditions, significant genetic variation was detected for allocation patterns and for compensatory ability. Furthermore, in the low nutrient treatment, resource allocation to storage was positively, genetically correlated both with compensatory ability and tolerance, and compensatory ability was positively, genetically correlated with tolerance. These results demonstrate that allocation of resources to storage provided a mechanism for tolerance. A negative genetic correlation between compensatory ability and plant growth when undamaged suggests that this tolerance mechanism entailed an allocation cost. Under high nutrient
conditions, genetic variation in tolerance also existed, but allocation patterns did not explain tolerance. The importance of distinguishing between tolerance and compensatory ability is evaluated.
Chapter III. Genotypic Variation in Defense and Tolerance in *Asclepias syriaca*

*(This is the part of the dissertation done at Litzsinger)*

**Introduction**

Plants have evolved almost countless ways to resist attack and damage by organisms that consume living plant material. Commonly, though, resistance to herbivores and pathogens is classified into two categories: defense and tolerance (Smith 1989, Rosenthal and Kotanen 1994). Defenses, including chemical and physical properties, allow plants to avoid damage by deterring plant enemies (Kennedy and Barbour 1992), whereas tolerance gives plants the ability to survive, grow, and reproduce in the presence of damage (Trumble et al. 1993). Clearly, to understand the evolution of resistance, one must understand both aspects of resistance--tolerance and defense.

Because a response to selection for increased resistance will not occur if genetically-based variation in resistance is lacking (Herms and Mattson 1992, Simms 1992), genetic variation in resistance must be quantified to determine whether resistance has the potential to evolve. A bioassay can be used to quantify genetic variation in defense (Simms 1992). Level of infestation (in the case of galling and sap-sucking insects) (Maddox and Cappuccino 1986, McCrea and Abrahamson 1987, Strong et al. 1993), intensity of attack by herbivores or pathogens (Burdon 1980, Burdon and Marshall 1981, Simms and Rausher 1987, Parker 1991, Simms and Triplett 1994, Fineblum and Rausher 1995), or proportion of plant tissue damaged (Marquis 1990, Sork et al. 1993, Simms and Triplett 1994, Stowe et al. 1994) can be used to measure plant susceptibility. For each measure, defense is gauged as the opposite of
susceptibility. Although the defense mechanism remains unknown when using a bioassay, the benefit of this approach is that it quantifies the level of defense realized by a plant.

To quantify genetic variation in tolerance, researchers have often examined the difference in fitness components between damaged and undamaged plants that are genetically related (Bohn et al. 1973, Ortega et al. 1980, Soper et al. 1984, Castro et al. 1988, Dale et al. 1988, Simms and Triplett 1994, Fineblum and Rausher 1995). In these cases, tolerance has been equated with compensatory ability (i.e., the difference in fitness components between damaged and undamaged plants (sensu Belsky 1986)). Although plant responses to damage may increase plant fitness, with plants that have greater compensatory ability having greater tolerance, tolerance is not necessarily determined solely by compensatory ability; traits that improve plant vigor (i.e., traits that increase plant fitness whether damage occurs or not) may also contribute to tolerance. Genotypes with a greater ability to acquire resources or a greater capacity to use resources would have greater vigor, and those differences could lead to genotypic differences in tolerance.

Provided that genetic variation in resistance does exist, evolution of resistance may still be limited by other constraints. Evolution of increased resistance may be constrained if increased resistance through defense decreases resistance through tolerance (Fineblum and Rausher 1995). When traits that contribute to defense and those that confer tolerance are both costly, genotypes that invest more in defense will have fewer resources to allocate to tolerance, while genotypes that have greater tolerance will have fewer resources to invest in defense (Simms and Triplett 1994). In these cases, a negative, genetic correlation between defense and tolerance would be indicative of the tradeoff.
Ecological tradeoffs (sensu Simms 1992) may also constrain the evolution of resistance. Ecological tradeoffs occur when the ability to respond to one ecological factor is limited by the necessary ability to respond to a second factor. One type of ecological tradeoff occurs when defense against one plant enemy is negatively related to defense against a second (Da Costa and Jones 1971, Marquis 1990, Linhart et al. 1989). In such cases, a resistance trait enhances fitness by limiting the effect of one plant enemy, but reduces fitness to the extent that it allows for increased damage by other herbivores and pathogens.

Allocation costs (sensu Simms 1992) can also constrain the evolution of resistance. In a resource-limited environment, either resistance factor (defense or tolerance) will entail an allocation cost if resources invested in the resistance mechanism diminish the resources available for growth and reproduction. In such cases, resistant genotypes would have lower growth and reproduction than non-resistant genotypes in the absence of herbivores and pathogens, but would be expected to have greater fitness than non-resistant genotypes in the presence of plant enemies.

Tolerance may entail an allocation cost in cases where responses to damage provide the ability to tolerate damage. Responses to damage that increase compensatory ability can require an allocation of resources to storage prior to damage (Richards 1984, Polley and Detling 1988, van der Meijden et al. 1988, Dyer et al. 1991, Schierenbeck et al. 1994). In such cases, ability to compensate for damage will require an investment of energy that cannot be allocated to growth or reproduction, and therefore will be costly. Conversely, allocation costs would not be expected for traits that increase tolerance through plant vigor because such characteristics would also confer greater fitness when plants remained undamaged. Allocation costs should be examined for compensatory ability as well as for tolerance because the cost of compensatory ability could be obscured by other, non-costly traits that provide tolerance.
The present study examines the potential for the evolution of resistance in *Asclepias syriaca*, the common milkweed. To investigate the potential for a response to selection, I quantified genetic variation in both defense and tolerance. Furthermore, I quantified costs of resistance by determining whether a tradeoff between tolerance and defense occurred and by examining whether increased defense against one enemy species involved a decrease in defense against a second plant enemy. In addition, to investigate whether allocation costs constrain the evolution of resistance, I determined whether tolerance and compensatory ability entailed allocation costs, and whether compensatory ability contributed to tolerance.

Methods

*Study Species*—*Asclepias syriaca* L. (Asclepiadaceae) is the most abundant and widespread milkweed in the northeastern United States, with an eastern border of Maine through Virginia and a western border of the Dakotas through Kansas (Woodson 1954). It is a disturbed-habitat perennial found mostly in old fields, in lightly grazed pastures and along roadsides (McCauley 1989). *A. syriaca* is obligately outcrossing, dispersing pollen in an aggregate via a pollinium (Morse 1982, Wyatt and Broyles 1990). This mode of pollination typically results in all seeds within a fruit sharing the same outcrossed, paternal parent (Broyles and Wyatt 1990).

Cardenolides (Na⁺-K⁺ ATP-ase inhibitors) are produced as both constitutive and inducible chemical defenses in *A. syriaca* (Malcolm 1991, Malcolm and Zalucki 1995). Despite the presence of cardenolides, *A. syriaca* can lose an average of 10% of its foliar tissues, with 100% defoliation sometimes occurring (Chapter 2). In addition to vertebrate herbivores such as deer and rabbits, which occasionally damage plants, a group of specialized insects consistently feed on *A. syriaca*. Many of these insect herbivores have evolved physiological adaptations which allow them to overcome and utilize cardenolides as a defense against their own predators.
In addition, plant pathogens also attack and damage *A. syriaca* (Flynn and Vidaver 1995). For the present study, levels of attack and damage by *Uromyces asclepiadis*--a rust fungus (Uredinales), *Danaus plexippus*--the monarch butterfly (Lepidoptera: Danaidae), *Liriomyza* sp.--a leaf-mining fly (Diptera: Agromyzidae), and *Aphis nerii*--an aphid (Homoptera: Aphididae), were quantified.

**Experimental Design**--To examine tolerance, I collected individual fruits from seven spatially separated clones in a population of *A. syriaca* (Eureka, Missouri) and from two clones in a second population of *A. syriaca* (Litzsinger Road Ecology Center--Ladue, Missouri) in the fall of 1993. Because I used individuals from two sites, estimates of genetic variation are maximal estimates for population-level variation. I considered all seeds from an individual fruit to be full-sibs, so my estimate of additive genetic variance is an upper bound that includes components caused by non-additive genetic variance and variance due to a common environment (Falconer 1989).

In April 1994, seeds from each family were nicked at their radical end and placed on moistened filter paper in 60 x 15 mm petri dishes for 12 days. Seeds that had initiated roots by the twelfth day were transferred to 7-cm pots containing sand. Plants were placed in the University of Missouri-St. Louis greenhouse, watered as necessary, and fertilized twice weekly with 20 ml (at 105 ppm nitrogen) of Peters 20-10-20 fertilizer for soilless media for 28 days. Plants were then potted into 10-cm pots filled with peat. Fertilizer was increased to 100 ml twice weekly. Forty-three days following seed germination, plants were moved to Litzsinger Road Ecology Center (Ladue, Missouri), where they were planted in a common garden.

Plants from each of the nine families were replicated seven to nine times for each of three damage levels (0% damage, 12% damage, and 25% damage) in the common garden. The
common garden was blocked into three subplots to help factor out environmental variation. Plants were randomly positioned within block, with each block receiving equal numbers of plants by family and damage level. Plant mortality caused by planting stress and exclusion of plants due to rabbit browse reduced the sample size from 237 to 176 plants.

Seventy-seven days after seed germination, I used scissors to damage plants artificially. I simulated the typical pattern of damage caused by vertebrate and insect herbivores of *A. syriaca*. For each plant receiving 12% damage, I removed the distal 50% of the leaf area for the leaves from the top fourth of the plant's canopy. For plants receiving 25% damage, I removed the distal 50% of the leaf area for the leaves from the top half of the canopy.

From the initiation of the experiment to 117 days following seed germination, herbivores and pathogens were excluded from plants. Chlorpyrifos was applied to the soil next to each plant to eliminate soil herbivores. Resmethrin, a synthetic pyrethrin, was applied weekly to all plants to eliminate above ground herbivores. Any observed herbivores were removed by hand. At day 117, as an estimate of tolerance, I estimated total leaf area of each experimental plant. Total leaf area was estimated using the following regression: \( \text{Leaf area (cm}^2\text{)} = -1823.14 + 14.36(\text{LL}) + 43.34(\text{NL}) \), where \( \text{LL} \) = area of largest leaf and \( \text{NL} \) = number of leaves \( (F_{df=2,26} = 273.6; P < 0.0001; \text{adjR}^2 = 0.95) \). I estimated the area of the largest leaf using the following regression: \( \text{LL} = -0.11055 + 0.71003(\text{LW}) \), where \( \text{LW} \) = leaf length x leaf width \( (F_{df=1,499} = 12460; P < 0.0001; \text{adjR}^2 = 0.99) \).

Following these growth measurements, and until the end of the season (179 days from seed germination), herbivores and pathogens were allowed to attack all plants, including controls. To quantify realized defense, genetic variation in plant damage/infestation was measured for *Uromyces asclepiadis*, *Danaus plexippus*, *Liriomyza* sp., and *Aphis nerii*. Defense
was then calculated as the opposite of damage/infestation. For *Uromyces*, proportion of leaf area damaged by rust was measured for 5 to 10 1-cm² randomly selected areas on each of three systematically selected leaves for each plant. A leaf from the top, a leaf from the middle, and a leaf from the bottom of each plant were collected to account for variation in damage due to leaf age. Defense against *Uromyces* was calculated as the complement of proportion of leaf area damaged (i.e., 1 - proportion leaf area damaged). For *Danaus* and *Liriomyza*, proportion of leaves damaged was calculated for each plant. Defense against each was quantified as the complement of proportion of leaves damaged. Defense against *Aphis* was measured as rank abundance of aphids per plant, where 4=no aphids, 3=few aphids (<<100), 2=many aphids (100), and 1=very many aphids (>>100).

Because fewer than one-fourth of the plants produced flowers and seeds (43 total), reproductive measures were not used to estimate genotypic differences in tolerance. In April of the following year, before plants sprouted new ramets, all rhizomes were harvested, oven dried at 100° C for a minimum of four days, and weighed. Rhizome mass was used to quantify tolerance for each family. Rhizome mass could not be adjusted to account for the decrease in growth caused by the resources invested into reproduction because plant size was positively correlated with flower and seed production, even within families. Alternatively, leaf area 117 days following seed germination could have been used to estimate genetic variation in tolerance because it estimates tolerance prior to the effects of herbivore and pathogen damage and flowering/fruiting. However, leaf area ignores the investment of resources in below-ground plant parts, whereas rhizome mass considers whole plant biomass. In any case, outcomes using rhizome biomass and those using leaf area were statistical equivalent both for estimating genetic variation in tolerance and for all genetic correlations. Therefore, tolerance was measured as the
rhizome mass of damaged plants. In addition, compensatory ability was calculated for each full-sib family as the difference in rhizome mass between damaged and undamaged plants.

**Statistical Analyses**—Estimates of defense against *Uromyces*, *Danaus*, and *Liriomyza* were arcsine square root transformed to create more normal distributions of data (Zar 1984). Rhizome mass was used as a dependent variable in models that examined the effects of genotype and damage treatment on plant tolerance. For all analyses, F-tests were based on Type III sums of squares provided by SAS PROC GLM (SAS Institute 1985). Damage treatment was considered a fixed effect, as was the effect of block. Family was considered a random effect, as were interactions with family. All genetic correlations were calculated with SAS PROC REG (SAS Institute 1985), using family means for trait values. 

**Genetic variation in resistance**—I performed a multivariate analysis of variance with defense against *Uromyces*, *Danaus*, *Liriomyza*, and *Aphis* as the four dependent variables. Provided that the effect of family was significant in the multivariate analysis of variance, I performed univariate analyses of variance with defense as the dependent variable to determine whether families differed in defense against each enemy species. Family and block were used as independent variables in these models. Damage treatment was not included in models because the treatment explained no significant variation in any model.

I performed an analysis of variance using rhizome mass as the dependent variable to examine for variation in tolerance among families. Plants which received 0% damage were excluded from these analyses because tolerance is measured as fitness when damaged. Original models included the effects of family, block, damage treatment, and the interaction between family and damage treatment; however, no significant differences were detected between 12% and 25% damage levels in any model, and no interaction between damage treatment and family
was significant in any analysis. Because pooling the data did not change the statistical results for any analysis, I pooled the 12% and 25% damage levels into a single class--damaged plants. To test for genetic variation in tolerance, the final model included family and block as independent variables. In addition, to determine whether there was a plant response to damage, I performed analyses of variance using leaf area and rhizome mass as dependent variables. Family, damage treatment (i.e., damaged and undamaged classes), and block were included as independent variables.

Costs of resistance--To determine whether tradeoffs between tolerance and defense occurred, I performed correlations between family mean tolerance and family mean defense. Correlations were performed only if significant genetic variation in both resistance factors existed. To ascertain whether increased defense against one species involved decreased defense against a second species, correlations were performed for family mean defense for pairs of plant enemy species. Again, genetic correlations were performed only when genetic variation in defense existed for both factors.

To determine whether allocation costs for tolerance occurred, a family mean correlation between tolerance and growth in the absence of damage was performed using rhizome mass as the dependent variable. Other factors that provide tolerance, such as traits that improve plant vigor, could mask an allocation cost caused by resource allocation patterns. To avoid this problem, I also performed a family mean correlation between compensatory ability and growth in the absence of damage. Because compensatory ability considers only plant responses to damage, confounding factors that provide tolerance are eliminated. In addition, to determine whether compensatory ability contributes to tolerance, a family mean correlation between
tolerance and compensatory ability was performed using rhizome mass as the dependent variable.

**Results**

*Genetic variation in resistance*—The multivariate analysis of variance that included defense against all four plant enemy species as dependent variables was significant (Wilks' $\lambda_{df=32,588} = 1.52; P=0.03$). Defense against *Uromyces* varied greatly, with some plants having 30% of their leaf area damaged by rust and others receiving less than 1% damage. Although defense against *Uromyces* was not affected by previous level of manipulated damage (on average, plants from each damage level lost an additional 7% -8% of their leaf area), families differed significantly in defense against *Uromyces* ($F_{df=8,163} = 2.12; P=0.04$), with families ranging from slightly less than 5% leaf area damaged to just greater than 10% damage.

There was also a broad range of defense against *Danaus*; some plants received no damage from monarch caterpillars, while others had more than 35% of their leaves damaged by *Danaus*. On average, 4% -6% of leaves were damaged per plant, regardless of experimental damage level, but families varied in defense against *Danaus*; the family that showed greatest defense had an average of 2% of its leaves damaged, while the family that was least defended had a mean of 7% of its leaves damaged. Genetic variation in defense against *Danaus* was almost significant ($F_{df=8,164} = 1.71; P=0.10$).

The percent of leaves damaged by *Liriomyza* ranged from 0% -9% among plants. Defense against *Liriomyza* was not affected by previous level of manipulated damage; plants from each level of manipulated damage had a mean of 1% of their leaves damaged by leaf
miners. Mean percent leaves damaged ranged from 0% -5% among families, but genetic variation in defense against *Liriomyza* was not detected ($F_{df=8,164}=1.31; P=0.24$).

Some plants were attacked by thousands of aphids, but most plants had few to no aphids on them. Rank abundance of *Aphis* did not differ among manipulated damage levels; rank abundance ranged from 3.4 to 3.5 among damage levels. Moreover, rank abundance of *Aphis* did not differ among families ($F_{df=8,163}=0.53; P=0.83$), with rank abundance ranging from 3.2 to 3.7 among families.

Significant genetic variation in tolerance was detected using rhizome mass as a growth measurement ($F_{df=8,104}=2.12; P=0.04$). When experimentally damaged, mean rhizome mass ranged from 37 to 68 grams among families (Figure 3-1). In addition, plants that were experimentally damaged (by removing either 12% or 25% leaf area) did not differ from control plants in total leaf area (Table 3-1; Figure 3-2A); however, damaged plants had decreased rhizome mass compared to undamaged plants (Table 3-1; Figure 3-2B).

**Costs of resistance**--Because significant genetic variation in defense was demonstrated only against *Uromyces*, tradeoffs between tolerance and defense were examined using only this pathogen species. Defense against *Uromyces* was negatively correlated with tolerance, but the genetic correlation was not significant ($F_{df=1,7}=0.83; P=0.39; r_g=-0.32$).

Genetic variation in defense was demonstrated only for *Uromyces*, but genetic variation in defense against *Danaus* was almost significant ($F_{df=8,164}=1.71; P=0.10$). Therefore, I performed a genetic correlation between defense against *Uromyces* and defense against *Danaus* to determine whether increased defense against one of these species involved decreased defense
against the other. This genetic correlation was positive and not significant ($F_{df=1,7} = 1.32; P=0.29; r_g=0.40$).

Tolerance was positively, genetically correlated with fitness of undamaged plants ($F_{df=1,7} = 9.08; P=0.02; r_g=0.75$); families that grew well when undamaged also grew well when damaged (Figures 3-1 and 3-3). Compensatory ability was negatively, genetically correlated with performance of undamaged plants ($F_{df=1,7} = 16.0; P=0.005; r_g=-0.83$), with families that showed greater compensation for damage growing less when they remained undamaged (Figures 3-1 and 3-4). Further, compensatory ability was not genetically correlated with tolerance ($F_{df=1,7} = 0.52; P=0.49$).

Discussion

**Genetic variation in resistance**--The present study demonstrated the existence of genetic variation in defense against a pathogen, as has been previously observed in other non-agricultural species (e.g., Burdon 1980, Burdon and Marshall 1981, Parker 1991, Simms and Triplett 1994). The failure of disease to develop on some genotypes, but to be visible on other genotypes is characteristic of a qualitative defense against pathogens (i.e., defense in which one or few genes for defense are responsible for deterring the pathogen) (Burdon and Marshall 1981). Genetic variation for defense against *Uromyces* existed for the families sampled in this study, but all plant families experienced infection to some degree. Thus, the genetic basis for defense against this rust fungus appears to be quantitative, not qualitative in nature.

Lack of detection of significant variation among families for defense against *Danaus* could be due to the method of estimating defense (i.e., measuring proportion of leaves damaged, as done in this case, could be less sensitive at detecting variation than measuring proportion of leaf area damaged, as done in the case of *Uromyces*). Still, the differences among families in
defense against Danaus provide evidence that genetic variation may exist for the quantitative traits that code for defense against this species. The underlying cause for variation in defense against Danaus in this study may be variation in cardenolide concentration and/or latex levels. For other Asclepias species, female monarch butterflies avoid ovipositing on plants that have high cardenolide concentrations (Oyeyele and Zalucki 1990, Zalucki et al. 1990). Furthermore, early instar survival of monarch caterpillars has been negatively correlated with cardenolide concentration (Oyeyele and Zalucki 1990, Zalucki et al. 1990, Zalucki and Brower 1992).

The lack of genetic variation in defense against Liriomyza and Aphis may also be due in part to the methods of estimating defense against these herbivores (i.e., measuring proportion leaves damaged and rank abundance, respectively). Alternatively, these two herbivore species may circumvent the plant defenses, eliminating the effectiveness of plant defenses. By feeding on the phloem of A. syriaca, Aphis avoids the high levels of cardenolides and the latex transported in laticifers. Similarly, by mining within the leaf blade, Liriomyza may be able to avoid the high levels of latex and cardenolides found within the latex-containing vessels.

In no case was defense against a plant enemy species affected by previous experimental damage. A. syriaca is known to respond to damage by inducing higher levels of cardenolides (Malcolm and Zalucki 1995); however, cardenolide concentration returns to constitutive levels within five days. If cardenolides are responsible for defense against herbivore/pathogen species in the present study, it is not surprising that damage treatment had no effect since experimental damage occurred 40 days prior to consumers accessing the plants.

Although defense may be an important means by which A. syriaca avoids damage, tolerance may also be important. Several of A. syriaca's herbivores have evolved physiological adaptations that allow them to feed on it, often even sequestering the plant-generated
cardenolides as a defense against their predators (Malcolm 1991). In addition, high levels of damage can also be inflicted by vertebrate herbivores (Chapter 2). Since significant genetic variation in tolerance exists in the studied population (Figure 3-1), *A. syriaca* could potentially respond to herbivore/pathogen selection pressure by increasing tolerance.

No differences in rhizome mass were detected between the 12% and 25% manipulated damage levels. This outcome suggests that levels of damage within this range have equivalent impacts. In addition, damaged plants had leaf area equal to undamaged plants (Figure 3-2A), suggesting that plants responded to damage by investing resources in growth. However, rhizome mass was less for damaged plants than undamaged plants (Figure 3-2B), demonstrating that the investment of resources into photosynthetic machinery by damaged plants was not enough to completely replace lost tissues. Furthermore, compensatory ability was not genetically correlated with tolerance, so plant responses to damage did not confer tolerance under the conditions of this experiment.

**Costs of resistance**—Although evolution of increased resistance can also be constrained by tradeoffs between defense and tolerance (Fineblum and Rausher 1995), no such tradeoff occurred between defense against *Uromyces* and tolerance to damage. Both the traits that confer defense and those that provide tolerance are expected to be costly if tradeoffs between defense and tolerance are to be found (Simms and Triplett 1994). However, tolerance to damage in this study was not costly (Figure 3-3), so it is not surprising that no such tradeoff was detected.

No ecological cost, in the form of defense against two consumer species being negatively correlated with each other, was detected for *A. syriaca*. Although such tradeoffs have been found in several studies (Da Costa and Jones 1971, Marquis 1990, Linhart et al. 1989), a defense trait may provide resistance against several consumer species, making defense against those
plant enemies positively correlated. The genetic correlation between *Danaus* and *Uromyces* was positive, but it was not statistically significant. This suggests either that traits that provide defense against each plant enemy are distinct or that other factors affected defense, obscuring the correlation between these species. For example, defense against *Danaus* may depend not only on genetic variation, but also on predator/prey interactions. Induction of cardenolides by *A. syriaca* has been hypothesized to cause increased predation by the predators of *Danaus* (Malcolm and Zalucki 1995). Early instar larvae may move from one leaf to another to avoid higher levels of cardenolides caused by locally-induced herbivore damage. While moving from leaf to leaf, caterpillars may be more greatly exposed to predators. Alternatively, infection by *Uromyces* could be affected by factors other than genetic variation in defense. For example, the rust may be facilitated by herbivores (other than *Danaus*) by providing an entrance for infection through the damage they inflict. In either case, the genetic correlation between defense against the two plant enemy species would be weakened.

No allocation cost for tolerance was detected; in fact, fitness of experimentally undamaged plants was positively, genetically correlated with tolerance (Figure 3-3). Because greater tolerance was correlated with greater performance when plants remained undamaged, genetic variation in traits that improve plant vigor appear provide tolerance, and evolution of greater tolerance should not be constrained by allocation costs.

Interestingly, although tolerance was not costly, compensatory ability entailed an allocation cost; fitness of experimentally undamaged plants was negatively, genetically correlated with compensatory ability (Figure 3-4). This result suggests that compensatory ability requires an allocation of resources to storage prior to damage, a result observed for other plant species (Richards 1984, Polley and Detling 1988, van der Meijden et al. 1988, Dyer et al. 1991,
Schierenbeck et al. 1994). Unlike studies that have equated tolerance with compensatory ability (Bohn et al. 1973, Ortega et al. 1980, Soper et al. 1984, Castro et al. 1988, Dale et al. 1988, Simms and Triplett 1994, Fineblum and Rausher 1995), the results of the present study suggest that selection should favor plants with lesser compensatory ability because plants with greater compensatory ability had lower fitness when undamaged and did not show greater tolerance to damage. Moreover, these findings suggest that traits which contribute to compensatory ability may not necessarily contribute to tolerance. Thus, equating compensatory ability with tolerance can cause erroneous perceptions as to which genotypes selection would favor.
Table 2-1. Insect herbivores of *Asclepias syriaca* that were observed in the field (modified from S.B. Malcolm pers. comm.). Herbivores listed all specialize on milkweeds (specialization may include other plant taxa that have cardenolides). Cardenolide column states whether cardenolides are sequestered by the insect. Cardenolide information taken from Duffey and Scudder (1972), Isman et al. (1977), Vaughan (1979), Cohen and Brower (1983), Nishio et al. (1983). Unknown = questionable or no information.

<table>
<thead>
<tr>
<th>Herbivore Species</th>
<th>Feeding Stage</th>
<th>Feeding Type</th>
<th>Appearance</th>
<th>Cardenolides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milkweed beetle</td>
<td>larva</td>
<td>root chewer</td>
<td>white</td>
<td></td>
</tr>
<tr>
<td><em>Tetraopes tetraophthalimus</em> (Coleoptera: Cerambycidae)</td>
<td>adult</td>
<td>leaf chewer†</td>
<td>aposematic</td>
<td>Yes</td>
</tr>
<tr>
<td>Monarch butterfly</td>
<td>larva</td>
<td>leaf chewer</td>
<td>aposematic</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Danais plexippus</em> (Lepidoptera: Danaidae)</td>
<td>adult</td>
<td>nectar feeder</td>
<td>aposematic</td>
<td>Yes</td>
</tr>
<tr>
<td>Milkweed leaf miner</td>
<td>larva</td>
<td>leaf miner</td>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td><em>Liriomyza sp.</em> (Diptera: Agromyzidae)</td>
<td>adult</td>
<td>unknown</td>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td>Oleander aphid</td>
<td>nymph</td>
<td>phloem sucker</td>
<td>aposematic</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Aphis nerii</em> (Homoptera: Aphididae)</td>
<td>adult</td>
<td>phloem sucker</td>
<td>aposematic</td>
<td>Yes</td>
</tr>
<tr>
<td>Large milkweed bug</td>
<td>nymph</td>
<td>seed feeder*</td>
<td>aposematic</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Oncopeltus fasciatus</em> (Hemiptera: Lygaeidae)</td>
<td>adult</td>
<td>seed feeder*</td>
<td>aposematic</td>
<td>Yes</td>
</tr>
<tr>
<td>Small milkweed bug</td>
<td>nymph</td>
<td>seed feeder*</td>
<td>aposematic</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Lygaeus kalmii</em> (Hemiptera: Lygaeidae)</td>
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<td>seed feeder*</td>
<td>aposematic</td>
<td>Yes</td>
</tr>
<tr>
<td>Dogbane tiger moth</td>
<td>larva</td>
<td>leaf chewer</td>
<td>aposematic</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Cycnia inopinatus</em> (Lepidoptera: Arctiidae)</td>
<td>adult</td>
<td>nectar feeder</td>
<td>aposematic</td>
<td>Yes</td>
</tr>
<tr>
<td>Milkweed tiger moth</td>
<td>larva</td>
<td>leaf chewer</td>
<td>aposematic</td>
<td>No</td>
</tr>
<tr>
<td><em>Euchaetias egle</em> (Lepidoptera: Arctiidae)</td>
<td>adult</td>
<td>nectar feeder</td>
<td>aposematic</td>
<td>No</td>
</tr>
<tr>
<td>Milkweed weevil</td>
<td>larva</td>
<td>seed borer</td>
<td>cryptic</td>
<td>No</td>
</tr>
<tr>
<td><em>Rhyssomatus lineaticollis</em> (Coleoptera: Curculionidae)</td>
<td>adult</td>
<td>leaf chewer</td>
<td>cryptic</td>
<td>No</td>
</tr>
<tr>
<td>Milkweed leaf beetle</td>
<td>larva</td>
<td>leaf chewer</td>
<td>aposematic</td>
<td>No</td>
</tr>
<tr>
<td><em>Labidomera clivicollis</em> (Coleoptera: Chrysomelidae)</td>
<td>adult</td>
<td>leaf chewer</td>
<td>aposematic</td>
<td>unknown</td>
</tr>
</tbody>
</table>
* by means of cell sucking
† also floral chewer
Table 3-1. Analyses of variance for plant growth. Leaf area and rhizome mass are the dependent variables. A significant effect of damage treatment indicates that plant growth was affected by experimental damage. F-tests are based on type III sums of squares provided by SAS PROC GLM (SAS Institute 1985).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Type III SS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leaf area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family</td>
<td>8</td>
<td>85199442</td>
<td>7.43</td>
<td>0.0001</td>
</tr>
<tr>
<td>Damage</td>
<td>1</td>
<td>499264</td>
<td>0.34</td>
<td>0.56</td>
</tr>
<tr>
<td>Block</td>
<td>2</td>
<td>12804406</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>164</td>
<td>237897738</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Rhizome mass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family</td>
<td>8</td>
<td>21688</td>
<td>4.42</td>
<td>0.0001</td>
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<tr>
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<td>2451</td>
<td>3.98</td>
<td>0.05</td>
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<tr>
<td>Block</td>
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<tr>
<td>Error</td>
<td>164</td>
<td>100564</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4-2. Analysis of covariance for root:shoot ratios across nutrient treatments.

Root:shoot ratio is the dependent variable, and LEAFAREA\textsubscript{70} is the covariate. A significant damage treatment demonstrates that growth following damage occurred at the expense of storage tissues in the roots. A non-significant nutrient treatment by damage treatment (N x D) interaction suggests that damage treatment did not affect resource allocation patterns differently across nutrient treatments. Satterthwaite approximate F-tests are based on type III expected mean squares provided by SAS PROC GLM (SAS Institute 1985).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
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<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family</td>
<td>14, 6.5</td>
<td>0.96</td>
<td>2.7</td>
<td>0.10</td>
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<td>Nutrient</td>
<td>1, 99.8</td>
<td>1.99</td>
<td>73.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>Damage</td>
<td>1, 17.8</td>
<td>0.16</td>
<td>7.8</td>
<td>0.01</td>
</tr>
<tr>
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<td>14, 14.1</td>
<td>0.35</td>
<td>1.2</td>
<td>0.34</td>
</tr>
<tr>
<td>F x D</td>
<td>14, 13.9</td>
<td>0.29</td>
<td>1.0</td>
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<tr>
<td>N x D</td>
<td>1, 17.7</td>
<td>0.00</td>
<td>0.0</td>
<td>0.91</td>
</tr>
<tr>
<td>F x N x D</td>
<td>14, 128</td>
<td>0.29</td>
<td>0.7</td>
<td>0.74</td>
</tr>
<tr>
<td>LEAFAREA\textsubscript{70}</td>
<td>1, 128</td>
<td>0.56</td>
<td>20.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>128</td>
<td>3.59</td>
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</table>
Unfortunately, these figures would not update under the new Word software:

**Figure 3-1.** Norms of reaction across damage classes for the nine families, using rhizome mass as the measure of fitness. For each family, tolerance to damage is represented by the rhizome mass when damaged. The difference between mass when receiving damage (12% or 25%) and mass when undamaged represents compensatory ability.

**Figure 3-2.** A. Leaf area of plants 42 days after the manipulated damage treatment. Leaf area did not differ between damaged and undamaged plants ($F=0.34; df=1,164; P=0.56$) (error bars = ±1 s.e.). B. Rhizome mass of plants. Rhizome mass of damaged plants (12% and 25%) was less than rhizome mass of control plants ($F_{df=1,164}=3.98; p=0.05$) (Error bars = ±1 S.E.).

**Figure 3-3.** Scatter plot representing the genetic correlation between fitness of undamaged plants and tolerance ($F_{df=1,7}=9.08; P=0.02; r_g=0.75$). Values plotted are family means.

**Figure 3-4.** Scatter plot representing the genetic correlation between fitness of undamaged plants and compensatory ability ($F_{df=1,7}=16.0; P=0.005; r_g=-0.83$). Values plotted are family means.
Literature Cited


Nishio, S., M.S. Blum, and S. Takahashi. 1983. Intraplant distribution of cardenolides in Asclepias humistrata (Asclepiadaceae), with additional notes of their fates in Tetraopes melanurus (Coleoptera: Cerambycidae) and Rhyssomatus lineaticollis (Coleoptera: Curculionidae). Memoirs of the College of Agriculture, Kyoto University 122:43-53.


